SWI2/SNF2 chromatin remodeling ATPases overcome polycomb repression and control floral organ identity with the LEAFY and SEPALLATA3 transcription factors

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Patterning of the floral organs is exquisitely controlled and executed by four classes of homeotic regulators. Among these, the class B and class C floral homeotic regulators are of central importance as they specify the male and female reproductive organs. Inappropriate induction of the class B gene *APETALA3* (*AP3*) and the class C gene *AGAMOUS* (*AG*) causes reduced reproductive fitness and is prevented by polycomb repression. At the onset of flower patterning, polycomb repression needs to be overcome to allow induction of *AP3* and *AG* and formation of the reproductive organs. We show that the SWI2/SNF2 chromatin-remodeling ATPases SPLAYED (SYD) and BRAHMA (BRM) are redundantly required for flower patterning and for the activation of *AP3* and *AG*. The SWI2/SNF2 ATPases are recruited to the regulatory regions of *AP3* and *AG* during flower development and physically interact with two direct transcriptional activators of class B and class C gene expression, LEAFY (LFY) and SEPALLATA3 (SEP3). SYD and LFY association with the *AP3* and *AG* regulatory loci peaks at the same time during flower patterning, and SYD binding to these loci is compromised in *lfy* and *lfy sep3* mutants. This suggests a mechanism for SWI2/SNF2 ATPase recruitment to these loci at the right stage and in the correct cells. SYD and BRM act as trithorax proteins, and the requirement for SYD and BRM in flower patterning can be overcome by partial loss of polycomb activity in *curly leaf* (*clf*) mutants, implicating the SWI2/SNF2 chromatin remodelers in reversal of polycomb repression.

Plant development occurs largely postembryonically (1), and, as a consequence, many cell-fate choices do not take place until long after embryogenesis. One example is flower development; in the rapid-flowering winter annual *Arabidopsis* the first flowers are formed 1 mo to 1 y after germination (2). Precocious activation of the floral homeotic genes required for flower patterning results in pleiotropic defects including poor seed set and is prevented by chromatin repression, which is faithfully inherited throughout cell divisions until the first flowers are formed (3–7). The repressive chromatin needs to be erased for flower patterning to be initiated in flower primordia. For class B genes, such as *APETALA3* (*AP3*), which are required for correct patterning of the showy petals and the male reproductive organs, activation of gene expression occurs in late stage 2 flower primordia in the cells that will give rise to whorls 2 and 3 of the flower (6). For class C gene *AGAMOUS* (*AG*), which is required for patterning both the male and the female reproductive organs, induction is observed in early stage 3 flowers in the cells that will give rise to whorls 3 and 4 (6).

The mitotically heritable chromatin repression of *AP3* and *AG* before flower formation is achieved by two polycomb complexes: polycomb repressive complex 1 (PRC1) and PRC2. PRC2 is responsible for trimethylation of lysine 27 of histone H3 (H3K27me3) (7, 8). Two putative H3K27 methyltransferases and PRC2 complex components, SWINGER and CURLY LEAF (CLF), act during vegetative and reproductive development of the plant sporophyte (7). H3K27me3 represses gene expression at loci like *AP3* and *AG* in part via recruitment of the second complex, PRC1 (7, 8). PRC1 further compacts chromatin and makes it refractory to transcription (5, 7–10). The PRC1 complex components LHP1 and EMF1 have direct roles in *AP3* and *AG* repression (7, 10–12).

How polycomb repression is overcome is not well understood (7, 13). One mechanism recently described is stress-induced reversal of polycomb repression by phosphorylation of serine 28 of histone H3 in mammals (14). Other candidates for this function are chromatin regulators that genetically act in opposition to polycomb repressors; these are collectively referred to as trithorax group (TrxG) proteins (8). In plants, several TrxG proteins have been identified with a role in floral homeotic gene expression (15–18). Thus far none of these were shown to be absolutely required for induction of floral homeotic gene expression, perhaps due to the presence of redundant activities. Sequence-specific binding proteins may also be important for reversal of polycomb repression. The plant-specific transcription factor LEAFY (LFY) and the MADS box transcription factor SEPALLATA3 (SEP3) play key roles in class B and class C gene induction, and their spatiotemporal expression overlaps with that of *AP3* and *AG* (6, 19–23). In addition, the homeodomain transcription factor WUSCHEL plays a role in activation of *AG* expression (24, 25).

A genetic screen for enhanced floral homeotic defects in the weak *lfy-5* mutant previously implicated a SWI2/SNF2 chromatin-remodeling ATPase as a positive upstream regulator of *AP3* and *AG* (26). SWI2/SNF2 chromatin-remodeling ATPases use the energy derived from ATP hydrolysis to alter the histone octamer–DNA interactions and thus the accessibility of genomic regions to transcription factors or the general transcriptional machinery in the context of chromatin (27). This is achieved by ejection or partial disassembly of one or two histone octamers in important regulatory regions or by sliding the intact histone octamer to a new position on the genomic DNA (27, 28). The chromatin-remodeling ATPases are central catalytic subunits of large multiprotein complexes (27, 28). Among the four SWI2/SNF2 ATPases present in the *Arabidopsis* genome (29), the closely related SPLAYED (SYD) and BRAHMA (BRM) are the best characterized (30, 31). Both *syd* and *brm* single-mutant flowers have mild and variable floral homeotic defects, yet their role in flower patterning is not well understood (26, 32).

Here we show that SYD and BRM are recruited to the *AP3* and *AG* regulatory regions at the onset of flower patterning. We find that SYD and BRM physically interact with the transcription factors LFY and SEP3 in plant cells, and that the recruitment of SYD to *AP3* and *AG* is dependent on LFY and SEP3. In

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addition, SYD and BRM are redundantly required for the activation of *AP3* and *AG* expression and for pattering of the central three whorls of the flower. The requirement for SYD and BRM can be overcome by reducing polycomb repression (removal of CLF activity). Finally, both SYD and BRM act as TrxG proteins. Our combined data suggest a mechanism for correct spatiotemporal TrxG protein recruitment to the *AP3* and *AG* loci and for triggering the reversal of polycomb repression.

Results

Floral Homeotic Defects in *syd-2 lfy-5* or *brm-101 lfy-5* Double Mutants Are due to Loss of *AP3* and *AG* Induction. To be able to assess the role of BRM in flower pattering, we crossed the *brm-101* null mutant to a weak allele of *lfy*, *lfy-5*. Like *syd-2* (26), *brm-101* strongly enhanced the floral homeotic defects of *lfy-5*: petals and stamens were replaced by sepal-like structures, and carpels were unfused with exposed ovules (Fig. 1*A*). When we examined the accumulation of the floral homeotic genes in the *syd-2 lfy-5* and *brm-101 lfy-5* double mutants by quantitative RT-PCR (qRT-PCR), we found that class B and class C floral homeotic gene expression was dramatically reduced relative to the *lfy-5* single mutant (Fig. 1*B*). Moreover, in situ hybridization revealed that both the class B gene *AP3* and the class C gene *AG* were indeed expressed at very low levels in stage 3 *syd-2 lfy-5* and *brm-101 lfy-5* flower primordia (Fig. 1*C*). Lack of *AP3* and *AG* up-regulation was also observed in the double mutants using reporter constructs (Fig. S1*A*). Because the defect in *AP3* and *AG* induction preceded the morphological defects in the double-mutant flowers, it is likely the cause and not the consequence of these morphological defects.

We next tested whether the defects in B and C up-regulation in *syd-2 lfy-5* and *brm-101 lfy-5* might be triggered by reduced expression of upstream activators or by increased expression of upstream repressors of these genes (6). We did not observe any expression changes for known upstream regulators that would support a causal role in the reduced *AP3* or *AG* induction (Fig. S1 *B–D*). Thus, the floral homeotic defects in *syd-2 lfy-5* and *brm-101 lfy-5* plants are likely due to the failure to up-regulate class B and class C floral homeotic genes, including *AP3* and *AG*.

SYD and BRM Are Required for *AP3* and *AG* Up-regulation. Consistent with their subtle floral homeotic phenotypes (Fig. S2*A*) (26, 32), the expression of the class B and class C floral homeotic genes was only slightly reduced in *syd* and *brm* single mutants compared with wild-type flowers (Fig. S2*B*). Notably, *brm-101* showed a stronger reduction in *AP3* levels, whereas *syd-2* exhibited a more severe reduction in *AG* levels (Fig. S2*B*). Since both *syd* and *brm* mutants strongly enhanced the floral homeotic defects of *lfy-5*, we hypothesized that SYD and BRM may have redundant roles in flower patterning. Because *syd brm* double-null mutants are embryonic lethal (33), we devised a strategy to generate conditional *syd brm* double mutants in flower primordia. We designed an artificial microRNA (aMIR) (34, 35) directed against *BRM*, which phenocopied the *brm* null mutant when constitutively expressed (*p35S::aMIRBRM*, Fig. S3*A*). When we expressed *aMIRBRM* from a flower primordium-expressed promoter (*pLFY::aMIRBRM*) (36), we observed a specific reduction of *BRM* expression in young flower primordia as well as mild floral homeotic defects similar to those observed in *brm* null mutant flowers (Fig. 2*A*; Figs. S2*A* and S3*B*).

The conditional double mutant, *syd-2 pLFY::aMIRBRM*, was viable and formed flowers with severe floral homeotic defects. Petals and stamens were converted into sepal-like organs, whereas carpels were defective or absent (Fig. 2*A*). Double mutants between the intermediate *brm-3* and the null *syd-5* allele also displayed reduced petal and stamen identity as well as carpel defects (Fig. S3*C*). Moreover, similar to *syd-2 lfy-5* and *brm-101 lfy-5* flower primordia (Fig. 1*C*), *syd-2 pLFY::aMIRBRM* flower primordia had greatly reduced *AG* and *AP3* expression (Fig. 2*B*). Thus, SYD and BRM are redundantly required for *AG* and *AP3* up-regulation and floral organ identity specification, despite displaying distinct preferences for *AG* and *AP3* induction, respectively, on their own.

To test whether the floral homeotic defects in *syd-2 pLFY::aMIRBRM* can be overcome by adding back AP3 or AG activity, we introduced *AG* expressed from a promoter that is not subject to regulation by SYD or BRM (*p35S::AG*) (3) into *syd-2 pLFY::aMIRBRM*. Indeed, *p35S::AG* rescued the stamen and carpel identity defects in the third and fourth whorl of *syd-2 pLFY::aMIRBRM* flowers (Fig. 2*A*). The formation of stamens, which requires both class B and class C activity, is likely attributable to the up-regulation of *AP3* by AG (37) in *syd-2 pLFY::aMIRBRM p35S::AG*. Presence of *p35S::AG* did not trigger a reduction in *pLFY::aMIRBRM* levels (Fig. S3*D*). In addition, *p35S::AG* caused transformation of first-whorl organs into carpel-like structures, as well as loss of second-whorl organs in *syd-2 pLFY::aMIRBRM*, as previously described for *p35S::AG* flowers (3). The combined data suggest that the floral homeotic defects in *syd-2 pLFY::aMIRBRM* are likely due to the failure to induce *AP3* and *AG* expression.

SYD and BRM Associate with the *AP3* and *AG* Loci. To test whether SYD and BRM may directly regulate *AG* and *AP3* expression, we probed binding of the chromatin-remodeling ATPases to the regulatory regions of these loci using chromatin immunoprecipitation (ChIP) with anti-SYD (38) and BRM (32) antisera. We assessed occupancy at the *AP3* and *AG* loci both before and during flower patterning using a synchronized flower induction system (*ap1 cal p35S::AP1-GR*) (39). Mock-treated *ap1 cal p35S::*

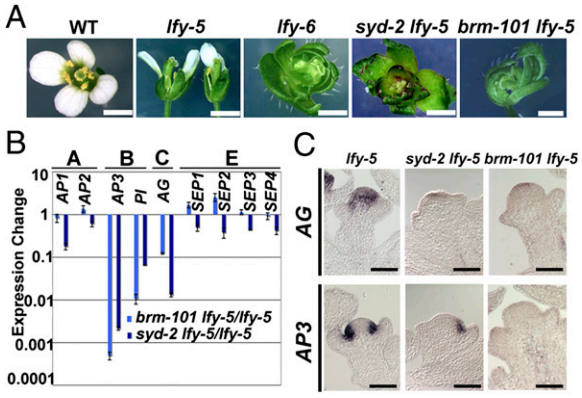

Fig. 1. SYD and BRM are required for proper floral homeotic gene expression. (*A*) *brm-101* enhances *lfy-5* floral homeotic defects. (Scale bars: 1 mm.) (*B*) qRT-PCR analysis of class A, B, C, and E floral homeotic gene expression in *syd-2 lfy-5* and *brm-101 lfy-5* double-mutant relative to *lfy-5* single-mutant inflorescences. Shown are the mean ± SEM. (*C*) In situ hybridization of *AP3* and *AG* expression in stage 3 flowers of *lfy-5*, *syd-2 lfy-5*, and *brm-101 lfy-5* mutants (Scale bars: 30 μm).

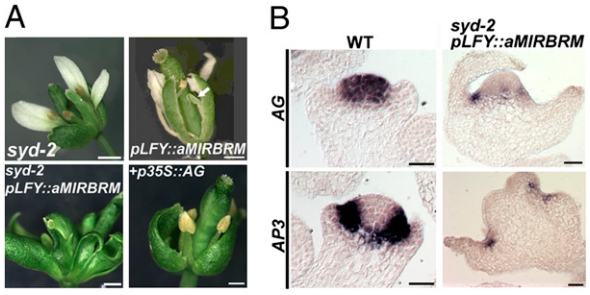

Fig. 2. SYD and BRM are required for *AG* and *AP3* induction. (*A*) Homeotic defects of *syd*-2, *syd-2 pLFY::aMIRBRM*, and *pLFY::aMIRBRM* flowers and rescue of the class C patterning defects of *syd-2 pLFY::aMIRBRM* by *p35S::AG*. (Scale bars: 0.5 mm.) (*B*) In situ hybridization of *AG* (*Upper* panels) and *AP3* (*Lower* panels) expression in stage 3 wild-type and *syd-2 pLFY::aMIRBRM* flowers. (Scale bars: 30 μm.)

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AP1-GR inflorescences are arrested before the initiation of flower patterning (39). A single steroid treatment triggers *LFY* induction, which peaks after 1 d, followed by full *AG* and *AP3* up-regulation at day 2 (Fig. 3*A*) (39). On the basis of ChIP, the occupancy of LFY, SYD, and BRM at *AG* and *AP3* regulatory regions was low before initiation of flower patterning, in mock-treated *ap1 cal p35S::AP1-GR* inflorescences (Fig. 3 *B–D*). Mirroring the time course of *AG* and *AP3* up-regulation, LFY binding to *AG* and *AP3* regulatory regions increased 1 d after induction with a further boost on day 2 (Fig. 3*B*). Like LFY, SYD and BRM strongly associated with the *AP3* and *AG* regulatory regions when flower patterning was initiated (Fig. 3 *B–D*). The temporal recruitment of SYD to the *AP3* and *AG* loci was very similar to that of LFY (Fig. 3 *B* and *C*), whereas BRM binding was strongest 1 d after AP1-GR activation (Fig. 3*D*). Consistent with the stronger effect of SYD on *AG* expression and BRM on *AP3* expression, SYD and BRM preferentially bound to the *AG* and *AP3* regulatory regions, respectively (Fig. 3 *C* and *D*). LFY, SYD, and BRM did not bind to a control heterochromatic region (*TA3*) (40) or to neighboring loci (Fig. 3 *B–E*). Hence, during the initiation of flower patterning, SYD and BRM were specifically recruited to *AG* and *AP3* regulatory regions.

SYD and BRM Physically Interact with LFY. LFY, SYD, and BRM are coexpressed in early stage 3 primordia (Fig. 4*A*), in the tissues and at the stage when *AP3* and *AG* expression is activated (6). In addition, all three proteins are recruited to regulatory regions of the *AP3* and *AG* loci at the onset of flower patterning. We therefore next probed for a possible physical association between LFY and SYD or BRM. In vitro pull-down experiments with the N-terminal protein-interaction domains (42) of SYD and BRM (SYDN and BRMN) and the recombinant LFY protein fused to the C terminus of GST (GST-LFY) revealed that SYDN and BRMN interacted with LFY. No interaction was detected when GST alone was used as bait or when an unrelated protein (GUS) was used as prey (Fig. 4*B*). The association between LFY and SYDN or BRMN was independently corroborated by three additional assays, yeast two-hybrid tests (Fig. S4*A*), bimolecular fluorescence complementation (BiFC; Fig. 4*C*), and coimmunoprecipitation (Fig. 4*D*). In BiFC, all transformed cells in which LFY and SYDN or BRMN were present showed YFP fluorescence. No signal was observed when an unrelated nuclear-localized protein was used (Fig. 4*C*), suggesting that the observed interactions were specific. Finally, we performed immunoprecipitation of GFP-SYD using anti-GFP antibody from nuclear extracts derived from two independent *pSYD::GFP-SYD* lines crossed into the *ap1 cal p35S:AP1-GR* background. We were able to detect the LFY protein in the immunocomplexes by Western blotting (Fig. 4*D*). When the GFP antibody was omitted, no LFY protein was detected. These findings suggest that LFY and SYD (as well as BRM) physically interact.

SYDN and BRMN contain two conserved regions typical of SNF2-type ATPases, a QLQ-rich region and an evolutionarily conserved helicase/SANT-associated (HSA) domain (29, 30). The HSA domain of mammalian and yeast SWI2/SNF2 ATPases is important for protein–protein interaction (42). GST-LFY pull-downs using serial SYDN and BRMN deletions revealed that two small evolutionarily conserved regions, the HSA domain and an adjacent motif, were important for the LFY interaction (Fig. S4 *B–D*). The QLQ domain, by contrast, was dispensable.

LFY Plays a Role in SYD Recruitment to *AP3* and *AG*. Given the physical interaction between LFY and SYDN or BRMN, we next tested whether recruitment of the SWI2/SNF2 ATPases to the floral homeotic loci was dependent on LFY. Because SYD binding to its target loci was more readily detectable than that of BRM (Fig. 3 *C*

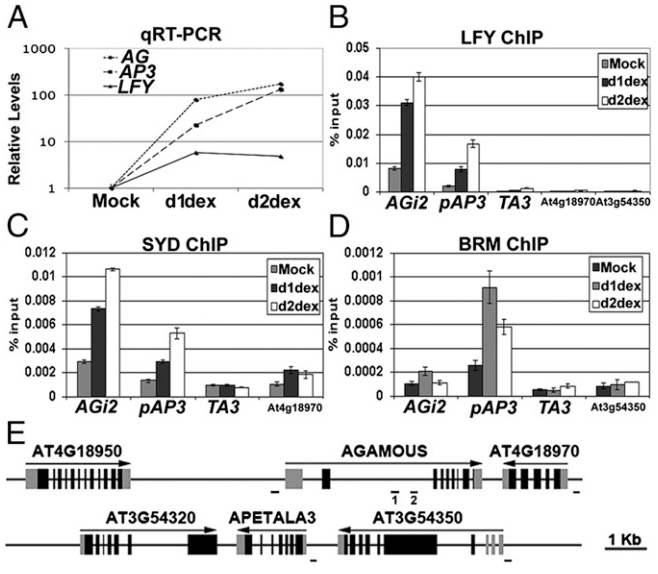

Fig. 3. Recruitment of SYD and BRM to *AP3* and *AG* loci. (*A–D*) Synchronous flower induction system (*ap1 cal 35S::AP1-GR*) (39). (*A*) *LFY*, *AG*, and *AP3* expression in mock-treated or dexamethasone-treated (dex; 1 μM) *ap1-1 cal-1 p35S::AP1-GR* inflorescences 1 or 2 d after induction. (*B–D*) ChIP assays using anti-LFY (*B*), anti-SYD (*C*), and anti-BRM antibody (*D*) in *ap1-1 cal-1 p35S::AP1-GR* inflorescences treated as in *A*. (*E*) Diagram of the *AG* and *AP3* genomic regions. Lines, gray boxes, and black boxes represent noncoding, untranslated, and translated regions, respectively. Arrows indicate the orientation of transcription. (*Lower*) Regions amplified in ChIP qPCR including the known LFY-bound region in the *AP3* promoter (pAP3) and in the *AG* intron (AGi2) (19).

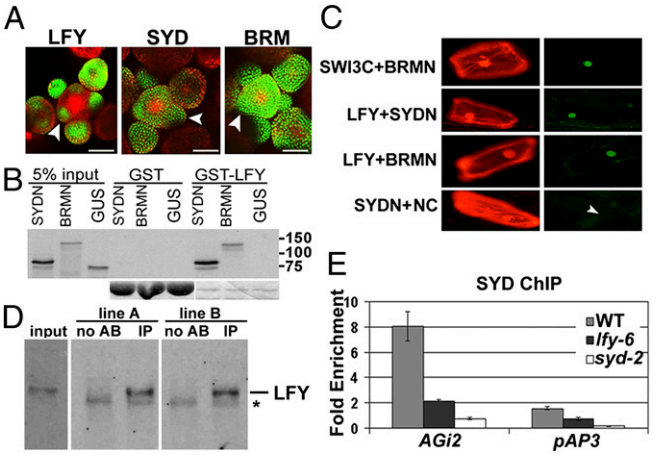

Fig. 4. LFY physically interacts with SYD and BRM. (*A*) Expression of a known translational reporter for GFP-LFY (41), as well as newly generated SWI2/SNF2 reporters (GFP-SYD and BRM-GFP; see *SI Materials and Methods* for details) in inflorescences on the basis of confocal microscopy (GFP fluorescence). Arrows point to stage 3 flowers. (*B*) Radiogram of physical interaction between LFY and the [^{35}S]methionine-labeled N-terminal domains of SYD or BRM (SYDN or BRMN) by in vitro GST pull-down assays (*Top*). The GUS protein served as a negative control. (*Bottom*) Level of GST or GST-fusion proteins. (*Right*) Approximate molecular mass in kilodaltons. (*C*) *In planta* interactions between LFY and SYDN or BRMN by BiFC. (*Left*) Transformed cells expressing *p35S:2xmCherry*. (*Right*) Interaction tests. SWI3C and an unrelated nuclear protein served as positive and negative controls, respectively (see *SI Materials and Methods* for details). (*D*) Immunoprecipitation from the nuclear lysates of two independent *ap1-1 cal-1 p35S::AP1-GR pSYD::GFP-SYD* transgenic lines (lines A and B) with mouse anti-GFP antibody. LFY protein in the immunoprecipitated complex ("IP"), no antibody control ("no AB"), and nuclear lysate ("input") was detected by Western blot using rabbit anti-LFY antibody. The asterisk indicates a nonspecific band present in the IP and control samples. (*E*) Anti-SYD ChIP in wild-type (WT), *lfy-6*, or *syd-2* inflorescences. Regions amplified in the *AP3* and *AG* loci are as in Fig. 3. Fold enrichment over that at the *TA3* retrotransposon locus. Means ± SEM are shown.

and *D*), we focused on SYD recruitment. To facilitate the comparison of multiple different genotypes, we normalized SYD genome occupancy over that at a negative control locus (*TA3*, Fig. 3 *B*–*D*). Relative to the wild type, SYD association with *AP3* and *AG* regulatory loci was strongly reduced in *lfy* null mutants (Fig. 4*E*). By contrast, LFY association with these loci was not dependent on SYD and BRM (Fig. S3*E*). These findings, combined with the observed physical and genetic interaction between SYD and LFY, and the similar timing of recruitment of the two proteins to *AP3* and *AG* loci upon initiation of flower patterning, strongly suggest that LFY plays a role in SYD recruitment to the *AP3* and *AG* regulatory regions.

Role for SEP3 in SYD Recruitment. Although SYD occupancy at the *AG* and *AP3* loci was reduced in *lfy* null mutants, it was still higher than the observed in *syd* null mutants (Fig. 4*E*). The residual binding of SYD to *AG* and *AP3* in *lfy*-6 raises the possibility that another factor contributes to SYD (and BRM) recruitment to the *AP3* and *AG* regulatory regions. Consistent with this idea, loss of SYD or BRM function further enhanced the floral homeotic defects of *lfy-6* null mutant flowers, resulting in reduced carpel identity and a less whorled phyllotaxis (Fig. 5*A*).

In addition, we predict that mutations in the additional SYD-recruiting factor should enhance the floral homeotic defects of *lfy* mutants. It is known that *ap1* mutants enhance the floral organ identity defect of *lfy-6* null mutants and cause a more complete loss of *AP3* and *AG* expression (Fig. 5*A*) (20). AP1 triggers floral homeotic gene expression primarily via up-regulation of *SEP3* (43, 44), and *SEP3* expression was very low in *ap1 lfy* double mutants (Fig. S5). Furthermore, *sep3* mutants enhanced the floral homeotic defects of the *lfy-1* null mutants: carpel identity was reduced and the organs in the flower were arranged in a spiral phyllotaxis (Fig. 5*A*). Finally, *AP3* and *AG* expression was essentially absent in *lfy-1 sep3-2* stage 3 flower primordia relative to *lfy-1* alone (Fig. 5*B*). Hence SEP3 is a possible candidate for a second transcription factor that may recruit SYD (and BRM) to *AP3* and *AG* regulatory regions. The enhancement of the *lfy* null mutant floral homeotic defect by *sep3* is consistent with prior findings that SEP3 acts both downstream of, and in parallel with, LFY (e.g., see ref. 19).

We therefore next examined whether SEP3 can physically interact with SYDN and BRMN. Recombinant GST-SEP3 was able to pull down SYDN and BRMN, but not a control protein (Fig. 5*C*). Furthermore, SEP3 interacted with BRMN and SYDN in plant cells on the basis of BiFC assays (Fig. 5*D*). To test whether SYD recruitment to *AG* and *AP3* is dependent on SEP3, we compared SYD binding to *AG* and *AP3* in wild-type, *lfy-1*, and *lfy-1 sep3-2* flowers by ChIP. We observed a slight reduction of the SYD binding to the *AP3* and *AG* loci in the *lfy-1 sep3-2* double-mutant flowers compared with wild-type and *lfy-1* flowers (Fig. 5*E*). The combined data are consistent with a possible role for SEP3 in SYD recruitment to the regulatory regions of the floral homeotic genes in the absence of LFY.

Mutual Antagonism Between SWI2/SNF2 and Polycomb Repressors. Because SYD and BRM were required for induction of *AP3* and *AG* during flower patterning (Fig. 2), we next asked whether this requirement was due to the known PRC2/PRC1-mediated repression of these loci (7, 13). To address this question, we examined the effect of partial loss of polycomb repression in *syd-2 pLFY::aMIRBRM*. This was achieved by introducing a null mutant for the PRC2 enhancer of Zeste homolog and predicted H3K27 methyltransferase CLF (*clf-2*) (45) into *syd-2 pLFY::aMIRBRM*. Intriguingly, many of the floral homeotic defects of *syd-2 pLFY::aMIRBRM* flowers were rescued in the triple mutant. *clf-2 syd-2 pLFY::aMIRBRM* flowers formed petal- and stamen-like organs and wild-type-looking carpels (Fig. 6*A*). Furthermore, loss of CLF activity caused a very strong increase in *AP3* and *AG* expression in *syd-2 pLFY::aMIRBRM* flower primordia on the basis of in situ hybridization (compare Fig. 6*A* and Fig. 2*B*) and qRT-PCR (Fig. 6*B*). The expression of *pLFY::aMIRBRM* was not altered in the *clf* mutant background (Fig. S3*D*). The data suggest that SYD and BRM activity are required for floral homeotic gene induction in the context of polycomb repression.

Because *clf* mutants could overcome the severe defects caused by the combined loss of SYD and BRM activity in young flower primordia, we next tested whether the *syd* mutant can also overcome the defects caused by partial loss of polycomb function in *clf-2*. During vegetative development, *clf-2* mutants have curled leaves due to ectopic expression of *AG* and *AP3* (45, 47). Removal of SYD activity in *clf-2 syd-2* double mutants led to loss of leaf curling and to formation of a flat lamina (Fig. 6*C*), as well as to a substantial (greater than 70%) reduction in *AP3* and *AG* expression (Fig. 6*D*). Similar results were obtained when we removed BRM activity (Fig. S6). The reciprocal ability of *clf* to overcome the floral homeotic defect in *syd-2 pLFY::aMIRBRM* mutants and of *syd* or *brm* to overcome the leaf curling and *AG*/*AP3* overexpression defects of *clf* mutants suggests that SWI2/SNF2 chromatin remodeling and polycomb repression antagonistically control chromatin-mediated expression of the shared direct target genes *AP3* and *AG*.

The ability of *syd* mutants to partially rescue the *clf* mutant defects suggests that SYD may act as a TrxG protein. To further probe this hypothesis, we examined the level of the CLF-mediated H3K27 trimethylation and of the activating histone mark H3K4me3 (7, 13) at *AG* and *AP3* regulatory regions. H3K27me3 levels were increased more than 70% at critical *AG* and *AP3* regulatory regions in *syd* mutants compared with the wild type (Fig. 6*E*). In addition, *clf-2 syd-2* displayed at least a 50% increase in H3K27me3 at these regions relative to *clf-2* (Fig. 6*E* and Fig. S7). The data are consistent with antagonistic roles for CLF and SYD in H3K27 trimethylation as well as *AP3* and *AG* expression levels and confirm a role for SYD as a TrxG protein. H3K4 trimethylation at the *AG* intron was slighty reduced in *syd* single mutants relative to the wild type (Fig. 6*E*), whereas *clf-2 syd-2* displayed a 40–60% reduction in H3K4me3 relative to *clf-2* at the relevant regulatory regions of both genes (Fig. 6*E* and Fig. S7). Thus, in addition to opposing polycomb repression of *AP3*

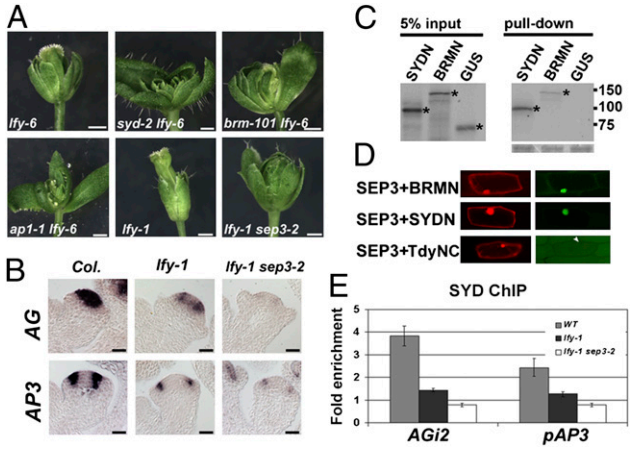

Fig. 5. SEP3 may contribute to SYD recruitment. (*A*) Floral homeotic defects in single and double mutants. Compare *syd-2 lfy-6*, *brm-101 lfy-6*, and *ap1-1 lfy-6* to *lfy-6* and *lfy-1 sep3-2* to *lfy-1*. *lfy-1* is in the Columbia ecotype, like *sep3-2*, and carries the same mutation as *lfy*-6. (Scale bars: 0.5 mm.) (*B*) In situ hybridization of *AG* or *AP3* expression in stage 3 wild-type (Columbia), *lfy-1*, and *lfy-1 sep3-2* flower primordia. (Scale bars: 30 μm.) (*C*) GST pull-down assays using GST-SEP3 and in vitro-translated radiolabeled SYDN, BRMN, or GUS. Relevant bands are marked by asterisks. The molecular mass is indicated on the right in kilodaltons. (*D*) BiFC experiments of SEP3 and SYDN or BRMN. (*Left*) Transfomation control (*p35S::2xmCherry*). (*Right*) YFP fluorescence in the same cells. Arrowhead points to the nuclei. (*E*) Anti-SYD ChIP in wild-type (WT; Columbia), *lfy-1*, and *lfy-1 sep3-2* inflorescences normalized as in Fig. 4*D*. Regions amplified in the *AP3* and *AG* loci are as in Fig.3. Shown is the mean ± SEM.

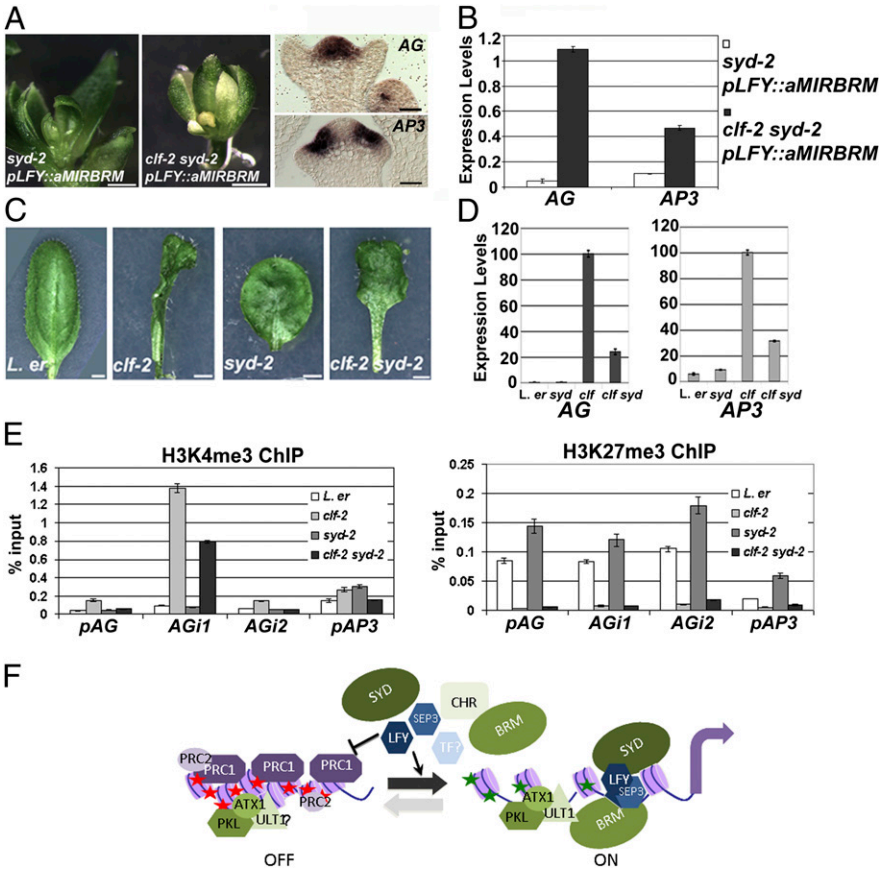

Fig. 6. Mutual antagonism between SWI2/SNF2 ATPases and polycomb repressors. (*A*) Loss of CLF activity in the *clf-2* null mutant restores the floral homeotic defects of *syd-2 pLFY::aMIRBRM* flowers and rescues *AP3* and *AG* expression in stage 3 *clf-2 syd-2 pLFY::aMIRBRM* flower primordia. (Scale bars: 0.5 mm for flowers and 30 μm for sections) (*B*) qRT-PCR of *AG* and *AP3* expression in 28-d-old *syd-2 pLFY::aMIRBRM* and *clf-2 syd-2 pLFY::aMIRBRM* inflorescences. (*C*) Loss of SYD activity in the *syd-2* null mutant restores leaf identity in *clf-2* mutants. Rosette leaves from 23-d-old plants are shown. (Scale bars: 0.5 cm.) (*D*) qRT-PCR of *AG* and *AP3* expression in 13-day-old wild-type (L. *er*), *clf-2*, *syd-2*, and *clf-2 syd-2* seedlings. The error bar represents the SEM. (*E*) Prevalent histone modifications in wild-type, *clf-2*, *syd-2*, and *clf-2 syd-2* seedlings. Anti-H3K4me3 and H3K27me3 ChIP was performed. For a map of the *AP3* and *AG* loci and regions amplified, see Fig. 3*E*. (*F*) Model for *AP3* or *AG* induction and reversal of polycomb repression. In seedlings and leaves as well as in nonexpressing tissues of the flower (OFF state), the following modifications or proteins are present: PRC1 and PRC2, high levels of H3K27me3 (red stars), and low levels of H3K4 (green stars) (this study) (10, 11, 18, 46, 51, 52). Two TrxG proteins, PKL and ATX1, are also thought to be present at this time, whereas occupancy of a third (ULT1) is not known (16–18). Floral homeotic gene activation (ON state) requires LFY, SEP3, and possibly additional transcription factors (TFs), which recruit SYD and BRM to the regulatory regions of *AP3* and *AG* (this study). ULT1 (18) and other chromatin factors (CHR) may also be recruited at this time. In addition, LFY directly represses expression (19) of the presumptive PRC1 complex component EMF1 (7). The combined activities result in loss of PRC2, H3K27me3, and PRC1 and lead to accumulation of H3K4me3 (this study) (16–19, 46).

and *AG*, SYD also contributes to activating chromatin modifications at these loci.

Discussion

We show that two SWI2/SNF2 chromatin-remodeling ATPases are redundantly required for formation of the male and female reproductive structures of the flower and for induction of *AP3* and *AG* expression. This important role has hitherto gone unnoted because of the embryo lethality of *syd brm* double-null mutants (33).

Our data further suggest that the balance of SWI2/SNF2 ATPase activity, on one hand, and of polycomb repression triggered by CLF, on the other hand, determines cell fate in the leaf and in flower primordia. SYD and BRM are required for *AP3* and *AG* induction in developing flower primordia when full polycomb repression is in effect. In the absence of *clf*, SYD and BRM are no longer absolutely required; in this case, sequence-specific transcription factors are presumably sufficient to activate *AP3* and *AG* expression. By contrast, *clf* leaves display ectopic expression of *AP3* and *AG* because the repressive chromatin at the *AP3* and *AG* loci is compromised (45, 46). The full ectopic expression has been shown to require the activity of TrxG proteins (15–18), including SYD and BRM (this study). Thus, CLF and SYD/BRM have opposite effects at the regulatory regions of the common target genes *AP3* and *AG*, with the dominant of the two activities ultimately determining the cell-fate choice. This idea is supported by the observation that a precocious increase in SYD activity, by premature removal of a negative regulatory domain, leads to upregulation of *AG* expression in leaves (48).

SYD and BRM physically interact with two tissue- and stage-specific activators of *AP3* and *AG*, the plant-specific transcription factor LFY, which is known to play a central role in class B and class C gene induction, and with the MADS box transcription factor SEP3, which is thought to act as a LFY cofactor (19–22, 49). Presence of these transcription factors is furthermore required for SYD recruitment to *AP3* and *AG* regulatory loci. A role of LFY in SYD recruitment is supported by three independent pieces of evidence: (*i*) LFY and SYD interact physically and genetically, (*ii*) they associate with the floral homeotic gene loci at the same time during flower development, and (*iii*) SYD binding to the *AP3* and *AG* regulatory regions is strongly reduced in *lfy* mutants. The observed interactions suggest a mechanism for spatiotemporal recruitment of SYD to the *AP3* and *AG* regulatory regions during initiation of flower patterning.

The finding that LFY recruits SYD (and perhaps BRM) for reversal of polycomb repression is consistent with prior observations. Direct LFY target genes at the time of flower patterning are enriched for genes subject to polycomb repression at earlier developmental stages (19). In addition, *de novo* motif analysis of regulatory regions bound by LFY at the time of flower patterning identified a *cis* motif linked to recruitment of polycomb complexes in *Drosophila* (8, 19). Finally, LFY directly down-regulates expression of the PRC1 complex component *EMF1* (Fig. 6*F*), a strong direct repressor of transcription at the *AP3* and *AG* loci with a role analogous to that of *Drosophila* posterior sex combs (5, 10, 19). A possible role for SEP3 in SYD recruitment fits well with its known function as a "gatekeeper" for the correct timing of class B and class C floral homeotic gene induction in conjunction with LFY (22, 49).

How is polycomb repression overcome at the *AP3* and *AG* loci during flower pattering? Although further investigations are required to fully address this question, the available data suggest that the LFY, SEP3, and perhaps additional transcriptional activators recruit SYD and BRM at the onset of flower patterning (Fig. 6*F*). Upon recruitment, SYD/BRM may eject one or more nucleosome to remove trimethylated H3K27 (and hence PRC1-docking sites). At the same time, increased activity of the TrxG proteins ARABIDOPSIS HOMOLOG OF TRITHORAX1

(ATX1; a H3K4me3 methyltransferase) and PICKLE (PKL; a chromodomain chromatin remodeler) is triggered; these two factors are thought to be present at the *AP3* and *AG* loci before their induction (16, 17) (Fig. 6*F*). A third TrxG protein, the SAND domain protein ULTRAPETALA1 (ULT1), is either recruited or activated at this stage (18). The full transition from the repressed to the activated chromatin state may involve recruitment of additional, as-yet-unidentified TrxG proteins (Fig. 6*F*). Rigorous test of this and additional possible scenarios awaits development and implementation of cell-type-specific assays for chromatin regulation in flower primordia.

Materials and Methods

Plants were in the Landsberg *erecta* background unless otherwise indicated and grown under 16-h cool-white fluorescent light at 22 °C. For all expression studies, the inflorescences of ~5-cm bolt plants were used. *ap1-1 cal-1 p35S::AP1-GR* inflorescences were treated with 1 μM of dexamethasone plus 0.015% of Silwet L-77 (39). RNA isolation, reverse transcription, and qPCR were as previously described (19). qRT-PCR was performed using three technical replicates for each biological replicate, and values were normalized to those obtained from *EIF4A* (At1g54270). The data from one representative biological replicate is shown. In situ hybridizations, GST pull-down, and BiFC experiments were performed as previously described (50). Coimmunoprecipitation was performed using nuclear extract prepared as described in ref. 48. ChIP experiments were performed as described in ref. 19. The amount of immunoprecipitated DNA after ChIP was computed by comparing the threshold cycle values between ChIP DNA and a dilution series of input DNA. The mean and SEM were calculated for three technical replicates of one to two biological replicates. See *SI Materials and Methods* for additional methods.

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